detergent head groups render Mistic extraordinarily resistant against unfolding, so that the protein largely retains its secondary structure even at urea concentrations higher than 7 M. Notably, for the application of Mistic as a fusion tag, polar or charged detergents have been found to be very successful in solubilizing the protein, which is in excellent agreement with our in vitro stability data. Thus, Mistic represents a unique model system to quantitatively assess the contribution of polar interactions to membrane-protein folding and extends the established view that hydrophobic interactions are the single most important contributors to the stability of membrane-interacting proteins.

1343-Pos Board B73

Building An Artificial Membrane Protein: Design, Expression and Characterization in Micelles and Lipid Vesicles

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High efficiencies and catalytic activities of natural oxido-reductases keep inspiring development of new enzymes with the goal to produce better catalysts, novel drug therapies, bioelectronics devices, bioremediation and alternative energy sources. However, the immense complexity of natural proteins and their very evident resistance and fragility to modification prevents scientists from deciphering what part of the protein structure is essential for reproducing the catalytic function itself apart from the many other demands made on a natural protein within a cell. To overcome this obstacle, we are designing general structural platforms (maquettes) that accommodate variety of oxido-reductase functions, including light energy harvesting, photochemical charge separation, oxygen transport, and oxidative metabolism. They also serve as models for understanding the fundamental properties of enzyme activity, stability, and folding. Here we will present a transmembrane maquette designed to form electron transfer chain across a lipid bilayer. This artificial membrane maquette contains four membrane spanning α-helices that are linked into a single chain. It has been expressed in high yields in inclusion bodies using E. Coli, purified and refolded in charged and uncharged detergent micelles, as well as in lipid vesicles. Circular dichroism studies revealed 70% α-helical nature in SDS and no melting in high temperatures and common denaturants, indicating its strong structural stability. We will present maquette assembly, stability, cofactor binding and redox characteristics in different membrane environments and how they compare to natural proteins.

1344-Pos Board B74

Folding Dynamics and Molecular Interactions of Outer Membrane Protein A

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The folding dynamics of outer membrane protein A (OmpA) has been characterized with several spectroscopic methods: circular dichroism (CD), tryptophan fluorescence, Förster resonance energy transfer (FRET), and ultra-violet resonance Raman (UVRR). These measurements provide information on secondary and tertiary structures, local environment, and intra- and inter-molecular interactions during the folding reaction. These results aim to elucidate molecular interactions that guide a protein as it inserts and folds into the bilayer, and we are motivated by the following questions: What are the mechanisms of folding dynamics in vitro, and are there specific tryptophan-lipid interactions that help guide membrane-protein interaction? Our results demonstrate that secondary and tertiary structures develop concurrently during protein insertion, followed by a long-time equilibration process that involves extension of transmembrane strands. Additionally, the vibrational data suggest that the tryptophan π-cloud is perturbed, possibly by the presence of a nearby charged group. These results enhance our understanding of membrane protein folding.

1345-Pos Board B75

Folding and Lipid Membrane Interactions of BamD, An Essential Component of the β-Barrel Assembly Machine from Escherichia Coli

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BamD (YfiO) [1] is an essential component of the β-barrel assembly machine of the outer membrane of Escherichia coli. BamD is synthesized in the cytosol and translocated across the cytopasmic membrane in unfolded form for processing and transport to the outer membrane by the LOL system [2]. To examine the interactions of BamD with lipid membranes and BamD folding, we have over-expressed BamD into the periplasm and isolated it from membrane fractions in unfolded form in 8 M urea. Circular dichorism spectroscopy indicated secondary structure formation in BamD upon rapid dilution of the denaturant urea in aqeuous buffer. In the absence of lipid bilayers, BamD displayed a lower content of α-helix structure. The CD-data indicated that native-like folding of BamD required the presence of lipid-membranes of phosphatidylglycerol and phosphatidylcholine. An increased content of phosphatidylglycerol facilitated BamD folding. Fluorescence spectroscopy demonstrated BamD binding to both zwitterionic and negatively charged lipid. The lipid/BamD stoichiometry was estimated to 17 phosphatidylglycerol/BamD and to 31 phosphatidylcholine/BamD.

[1] Malinverni JC, Werner J, Kim S, Sklar JG, Kahne D, Misra R, Silhavy TJ (2006) YfiO stabilizes the YaeT complex and is essential for outer membrane protein assembly in Escherichia coli. Mol Microbiol 61:151-164.
[2] Narita S, Tokuda H (2010) Sorting of bacterial lipoproteins to the outer membrane by the Lol system. Methods Mol Biol 619:117-129.

1346-Pos Board B76

Influences of the Hydrophobic Environment on the Structure and Function of Membrane Proteins and Development of Innovative Surfactants Called Amphipols

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Membrane proteins (MPs) exhibit a broad range of activities, which are crucial for cell survival. Understanding their molecular mechanisms generally requires their extraction out of membranes and their purification. Solubilization and isolation are usually carried out using detergents, which disrupt the membrane and adsorb onto the hydrophobic surface of the transmembrane domain of MPs, keeping them water-soluble. Detergents, however, tend to inactivate most MPs more or less rapidly, because they compete with stabilizing intra- and intermolecular interactions. The extent of this problem varies from one detergent to another but also considerably depends on the nature of MPs, creating a bias in our understanding of their structure and function in favor of the most robust MPs. Specially designed amphipatic polymers called 'amphipols' (APols) have been developed with the view of improving the stability of MPs in aqueous solutions. The first APols to have been synthesized comprise a poly-acrylic acid backbone onto which octylamine and isopropylamine side chains have been randomly grafted. The solution properties of APols and of the complexes they form with MPs have been investigated in some details (1). It has been demonstrated that APols are promising surfactants for biochemical and biophysical studies of MPs, because they form with MPs small and compact water-soluble complexes while improving the stability of the MPs they interact with. The rich chemistry of APols also allows modifications and labeling, generating a library of molecules that expand the scope of APol applications in both basic and applied research. Among the many novel surfactants developed to replace classical detergents, APols present the advantage of being remarkably easy to use (http://www.ibpc.fr/popot/amphipol/).

(1) Popot et al., Amphipols from A to Z (2011) Annu. Rev. Biophys. 40:379-408.

1347-Pos Board B77

Membrane-Enabled Dimerization of the Intrinsically Disordered Cytoplasmic Domain of ADAM10

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The intrinsically disordered protein region is widely distributed in native proteins, particularly in the cytoplasmic domain of many transmembrane receptors. These cytoplasmic domains are unstructured when expressed in isolation, but could acquire stable secondary structure when binding to a broad range of partners. The cytoplasmic domain of ADAM10, a transmembrane metalloprotease expressed in various tissues and cells, was recently suggested to mediate the homo-dimerization of ADAM10. But the underlying molecular basis remains unclear. Here, we show that a recombinant cytoplasmic domain of ADAM10 (ADAM10C) was unstructured as judged by its susceptibility to limited trypsin digestion and its circular dichroism spectrum. However, a recombinant protein consisting of transmembrane-cytoplasmic domains of ADAM10 (ADAM10TC) exhibited greater resistance to trypsin digestion, with the cytoplasmic sequence taking on significant α-helical structure. Further fluorescence resonance energy transfer and crosslinking analysis demonstrated that ADAM10TC, but not ADAM10C, formed tight homodimer in dodecylphosphocholine micelles. Placing the transmembrane and cytoplasmic domains of ADAM10, but not the transmembrane domain alone, in their native orientation in the inner membrane of E. coli produced strong homodimerization signal in the AraTM assay. Preliminary mutagenesis analysis further identified the juxtamembrane region of the cytoplasmic domain as important to dimerization. Overall, these results demonstrate that only upon adjoining the transmembrane domain does the cytoplasmic domain of ADAM10 take on α-helical conformation and form homodimer in the membrane. This is the first report of a transmembrane domain enabling dimerization of an adjacent intrinsically disordered sequence, which has general implications on the structure and function of the cytoplasmic domain in a transmembrane receptor.